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CHEMICAL COMPOSITION AND ANTIOXIDANT ACTIVITY OF ESSENTIAL OIL FROM *GLYCOSMIS PENTAPHYLLA*

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ABSTRACT

Several studies are going on worldwide directed towards finding natural antioxidants from plant origin which possess strong antioxidant activity. The aim of the present study was to find out chemical composition, radical scavenging activity and antioxidant activity of essential oil from *Glycosmis pentaphylla*. Essential oil was extracted from fresh leaves of *Glycosmis pentaphylla* by hydrodistillation and their chemical composition was determined by GC-MS analysis. Antioxidant activity of essential oil was examined by three different methods, 2, 2- diphenyl-1-picrylhydrazyl (DPPH), reducing power and phosphomolybdenum assay. Essential oil showed that it contains fifty four bioactive phytochemical compounds; among them, the major compounds are benzaldehyde oxime (15.66 %) caryophyllene oxide (7.47 %) aromandendrane (0.30 %). Essential oil of *G. pentaphylla* leaves showed the excellent antioxidant and radical scavenging properties. We have documented the promising antioxidant potential of essential oil from leaves of *Glycosmis pentaphylla*, which could be considered as a potentially alternative source for developing new drugs.

KEYWORDS: *Glycosmis pentaphyll*a, Volatile Oil, Antioxidant Activity, Radical Scavenging Activity, Reducing Power Assay, GC-MS

INTRODUCTION

The processes of oxidation are intrinsic in the management of energy of all living organisms and are, therefore, kept under strict control by several cellular mechanisms (Halliwell and Gutteridge, 2007). However, the production of excessive free radicals and the antioxidant protection due to unbalanced mechanisms result in the onset of numerous diseases and accelerate ageing. The antioxidants of low molecular weight are considered as possible protection agents reducing oxidative damage of the human body, when the internal enzymatic mechanisms fail or are inadequately efficient (Halliwell, 1995). Oxidation mediated by free radical reactions is also responsible for the rancidity of unpreserved food rich in unsaturated fatty acids and the natural antioxidants are suggested as a superior alternative for the synthetic ones such as BHA or BHT (Li *et al*., 2008). Therefore, there is a growing interest day by day in the substances exhibiting antioxidant properties, which are supplied to humans and animals as food components or as specific preventative pharmaceuticals (Sarikurrkcu *et al*., 2009). The plant kingdom is a good source to produce a wide range of natural antioxidants. However, still there is not enough knowledge and data about the practical usefulness of most of them. Groups of secondary plant metabolites, antioxidant, phenolics, and flavonoids are commonly found in various fruits, vegetables and herbs and they have been shown to provide a fruitful defense against oxidative stress from oxidizing agents and free radicals (Sarikurkcu *et al*., 2009; Matkowski , 2006; Antolovich *et al*., 2000) .

Glycosmis pentaphylla is an odorous shrub found throughout tropical and sub-tropical Himalaya, ascending up to an altitude of 2300 meter above mean sea level (Figure 1). The plant grows naturally in Sikkim, from the Sutlej River in the Northwest Southward to upper Assam and in Travancor and Malaca (India). It is also native to the Malay Archepelago, China, The Phillipine Islands, Bornea and Australia (Shastri, 1980; Chopra *et al*., 1992). It has been used as food, medicine and also for the treatment of fever, liver complaints, cough, rheumatism, anemia, jaundice and certain other diseases (Mohammed *et al* 2010).. The whole plant of *G. pentaphylla* contains alkaloids, flavonoids, terpenes and sterols.

Figure 1: Whole Plant of Glycosmis Pentaphylla

In this context, the aim of this study is to identify the chemical composition of *G. pentaphylla* essential oil and to evaluate the antioxidant and radical scavenging properties of the same. The results of the present study would be useful in promoting research aiming towards the development of new drugs from bioactive compounds derived from indigenous plant sources.

MATERIALS AND METHODS

G. pentaphylla *plant leaves were collected from Kalpakkam, Kanchipuram District, Tamil Nadu, India during the month of January, 2013.* G. pentaphylla *was identified by (Voucher No: 2000) Prof. P. Jayaraman, Plant Anatomy Research Centre (PARC), West Tambaram, Chennai* – 45.

Distillation of Essential Oils

Fresh leaves of *G. pentaphylla* were subjected to hydrodistillation using a modified Clevenger-type apparatus for 3 hours (Cheng *et al*., 2005). The yield was averaged over four experiments and calculated according to dry weight of the plant material. Essential oil was stored in an air-tight container prior to analysis by Gas Chromatography Mass Spectrometry (GC-MS).

Gc-Ms Analysis

The composition of the essential oil was determined using an Agilent 7890A Gas Chromatography Mass Spectroscopy instrument. Oxygen-free nitrogen was used as a carrier gas and hydrogen was used for the flame. The GC conditions used were as follows: capillary column: fused silica (Polydimethylsiloxane 0.25 µm film thickness); temperature program: 70ºC (2 min¹), 70-230º C (3 min¹), 230-240ºC (5 min¹), 270ºC (5 min¹); carrier gas, held at 5 bar, linear velocity of 20 cm min¹; injection port split less at 250ºC; injection volume, 0.1 µL. The MS conditions were as follows: ionization EI at 70 eV; m/z range, 30-300ºC; scan rate 1 sec ¹; ionization chamber at 180ºC; and transfer line at 280ºC. The identification of the essential oil constituents was done based on a comparison of their retention times and these

constituents were further identified and authenticated using MS data compared to the NIST mass spectral library (Adams).

Free Radical Scavenging Effect

The DPPH free radical scavenging effect was assessed by the method of Kondo and co-authors (Kondo *et al*., 2002). Various volumes of (20, 40, 60, 80 and 100 µl) of the stock essential oil solution (1 mg/ml) dissolved in ethanol were removed and placed into vials to give final concentrations (20, 40, 60, 80 and 100 µg/ml). Briefly, 0.1 mL of each essential oil at different concentrations (Diluted in ethanol) was added to 2 mL DPPH (0.21 mM in 95% ethanol). The mixture was shaken, left for 60 min. at room temperature in the dark and the absorbance was measured at 517 nm in a spectrophotometer. The percentage of DPPH inhibition was calculated using the following equation:

$$\text{Percentage of inhibition} = (A_{control} - A_{sample})/A_{control} \times 100$$

where $A_{control}$ is the absorbance of the control reaction (Blank with 0.1 mL ethanol and DPPH). A_{sample} is the absorbance of the sample reaction (0.1 mL essential oil diluted in ethanol and DPPH). Ascorbic acid was used as a reference standard.

Total Antioxidant Activity

The total antioxidant capacity of essential oil of *G. pentaphylla* was evaluated by the spectrophotometric method (Prieto *et al*., 1999). An aliquot of 0.3 ml of sample solution (100 µg/ml) was combined with 3 ml of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4mM ammonium molybdate). The tubes were capped and incubated in a boiling water bath at 95 ˚C for 90 minutes. After the sample has cooled to room temperature, the absorbance of the aqueous solution of each was measured at 695 nm against blank. A typical blank solution contains 3 ml of reagent solutions and appropriate volume of the same solvent used for the sample and it was incubated under same condition as rest of the sample. For the samples of unknown composition water soluble antioxidant capacity was expressed as equivalents of ascorbic acid (mg/g).

Reducing Power Assay

The reducing power of essential oil was determined according to the method of Oyaizu, 1986. The concentration of the sample ranged from (20 to 100 µg/ml). 0.5 ml sample made to 1 ml with ethyl acetate and mixed with 2.5 ml of Phosphate buffer (0.2 M, pH 6.6), 2.5 ml 1% Potassium ferricyanide [$K_3Fe(CN)_6$]. The mixture was incubated at 50 °C for 20 minutes and centrifuged at 5000 × g after addition of 2.5 ml of 10 % trichloroacetic acid. A 2.5 ml aliquot of upper layer (supernatant) was collected and mixed with 0.5 ml of 0.1 % ferric chloride. The absorbance was measured at 700 nm against a blank using UV-Vis spectrophotometer. Ascorbic acid at various concentrations was used as a standard. Increase in absorbance was directly correlated to increase in reducing power.

RESULTS AND DISCUSSIONS

Yield Percent and GC-MS Analysis

The essential oil obtained from fresh leaves of *G. petaphylla* by hydrodistillation yielded 0.1% per kg of fresh leaves (Figure 2). The oil phase was separated and dried over anhydrous sodium sulphate and kept in the dark glass bottle at 4˚C for further analysis. Results of GC/MS analysis showed 54 compounds (Table 1) in the essential oil of *G. pentaphylla*. These compounds were identified through Gas chromatography attached with MS. The mass spectra of these compounds were matched with those found in NIST/NBS spectral database. The principle compounds were bicyclo [6.1.0]

non-1-ene (18.93 %), benzaldehyde oxime (15.66 %), caryophyllene oxide (7.47 %), followed by 3,4-dimethyl-2-prop-2-enyl-2,5-dihydrothiophene, 1,1-dioxide (6.43 %), bicyclo [5.1.0] octane (4.74 %), 1,4-Dimethyl-8-isopropylidenetricyclo [5.3.0.0 (4,10)] decane (4.69 %), 3H, azepine, 2-methoxy (4.20 %), adamantane (4.17 %), <beta> panasinsene (3.11 %) and <beta> pinene (3.15 %) and their activity were mentioned (Table 2). Many phytomedicines exert their beneficial effects through the additive or synergistic action of chemical compounds that act at single or multiple target sites (Adwan *et al*., 2006). It is also possible that the minor components such as Caryophellene oxide, Beta Pinene, Caryophellene, Gamma terpinene might be involved in some type of antioxidant synergism with other active components of essential oil, as evident by the work of Marino, *et al*., (2001). Caryophyllene oxide, alpha terpineol found to possess antioxidant activity (Cavar *et al*., 2008; Chavan *et al*., 2010). In that sense for biological purposes, it is more informative to study on entire oil rather than some of its components because the concept of synergism appears to be more meaningful (Cal, 2006).

Radical Scavenging Assay

DPPH is a stable free radical that shows maximum absorbance at 517 nm. When DPPH radicals encounter a proton-donating substrate, such as an antioxidant, the radicals are scavenged and the absorbance is reduced (Shimada et al., 1992). The decrease in absorbance is taken as a measure of radical- scavenging activity. This is a widely used method to investigate the scavenging activity of some natural compounds. The free radical scavenging activity of the essential oil was done and the obtained result was compared with standard antioxidant, gallic acid. Figure 3 depicted the DPPH free radical scavenging activity of leaves of *G. pentaphylla* essential oil. The percentage of inhibition of DPPH free radical were observed as 70.71% whereas for gallic acid 83.82% at the concentration of 200 µg/ml. The IC_{50} value calculated for the essential oil was found to be 21. 92 µg/ml whereas for standard gallic acid, it was found to be 3.5 µg/ml.

Antioxidant Activity by Phosphomolybdenum Method

The total antioxidant capacity of essential oil from various parts of *G.P* was measured spectrometrically through phosphomolybdenum method, which is based on the reduction of Mo (IV) to Mo (V) by the sample analyte and subsequent formation of green phosphate compounds with a maximum absorption at 695 nm (Prieto *et al*., 1999) The antioxidant capacity of essential oil of *G.P* was found to be 0.260.

Reducing Power Assay

The reduction of Fe^{3+} is often used to measure electron donation, which is an important mechanism (Hinneburg et al., 2006). We determined the ability of essential oil to reduce Fe^{3+} to Fe^{2+} by measuring the formation of Pearls Prussian blue at 700 nm (Amarowicza *et al*., 2004). The reducing capacity of essential oil from leaves of *G.P* was found to be 1.372 whereas ascorbic acid was found to be 1.834 at the concentration 100 µg/ml (Figure 4). The reducing power of essential oil increases with increase in concentration of the sample. The essential oil from leaves of *G.P* was found to be most potent reductant which shows that the essential oil can react with free radicals to convert them to more stable products and thereby terminate radical chain reaction. The reductive potential may be related to the presence of phenolic compounds, due to hydroxyl substitutions in the aromatic ring, which possess potent hydrogen donating abilities, as described by Shimada, (1992). Antioxidant properties of terpinene were reported previously (Ruberto and Baratta, 2000).

CONCLUSIONS

Our results suggested that the essential oil of *G.P* can be utilized as an effective and safe source of natural antioxidants with consequent health benefits. It is proposed, that the beneficial effects of essential oil from *G.P* in traditional medicine results from their reducing ability towards reactive oxygen species. Besides their strong antioxidant activities, their low toxicities, wide distributions and medicinal functions all make them promising sources of natural antioxidants and other bioactive compounds in food and pharmaceutical industries. In the future, the specific compounds with high antioxidant capacities should be isolated, purified and identified from these plants to further develop natural antioxidants, which will be employed to treat these diseases in association with reactive oxygen species, such as cancer, atherosclerosis, coronary heart diseases and diabetes.

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APPENDICES

Figure 1: Whole Plant of Glycosmis Pentaphylla

Figure 2: Fresh Leaves *of Glycosmis pentaphylla* and its Essential Oil (Hydrodistillation)

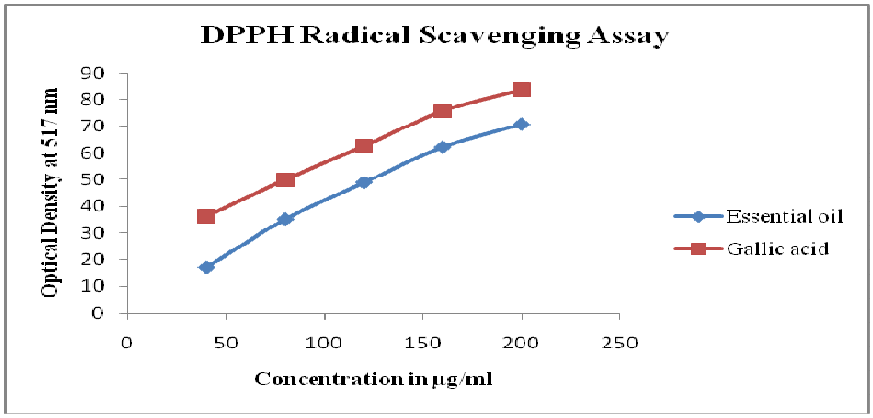

Figure 3: Radical Scavenging Activity of Essential Oil from *G. pentaphylla* by DPPH Assay

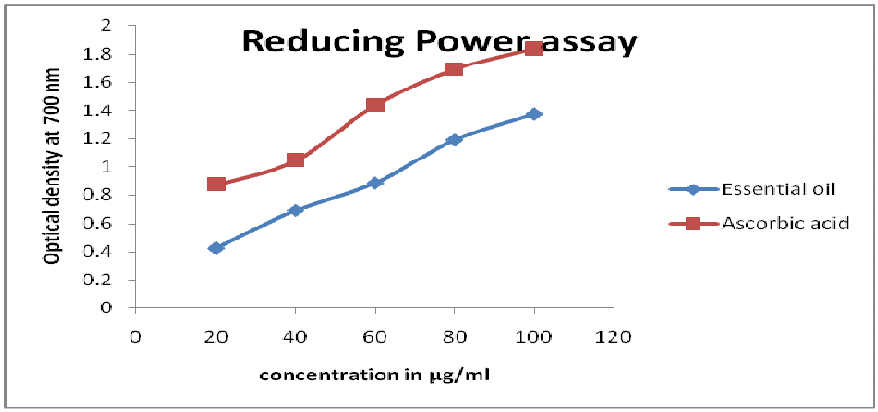

Figure 4: Reducing Ability of Essential Oil from *G. pentaphylla*

Table 1: Gas Chromatography Mass Spectrometry for Essential Oil from Leaves of *G. pentaphylla*

Sl. No	Retention Time	Area %	Compounds	Molecular Formula	Molecular Weight
1.	3.929	3.15	Beta-Pinene	$C_{10}H_{16}$	136.24
2.	4.176	0.06	Camphene	$C_{10}H_{16}$	136.23
3.	4.670	0.12	Cyclohexane,4-methylene-1-(1-methylethyl)	$C_{10}H_{18}$	138.24
4.	4.873	1.39	Santolina triene	$C_{10}H_{16}$	136.23
5.	5.149	2.27	1-Hepten-3-yne	C_7H_{10}	94.15
6.	5.251	0.29	(+)-3-Carene	$C_{10}H_{16}$	136.23
7.	5.367	0.09	Alpha terpinene	$C_{10}H_{16}$	136.23
8.	5.687	15.66	Benzaldehyde, oxime	C_7H_7NO	121.39
9.	5.774	4.74	Bicyclo[5.1.0]octane, 8methylene	C_9H_{14}	122.20
10.	5.919	2.43	Tricyclo[4.1.0.0(2,7)]heptane	C_7H_{10}	94.15
11.	6.108	0.37	Gamma-Terpinene	C10H16	136.23
12.	6.616	0.34	Terpinolene	$C_{10}H_{16}$	136.23
13.	6.776	0.11	1,6-Octadien-3-ol, 3,7-dimethyl	$C_{10}H_{14}O$	154.24
14.	7.052	0.29	1,4-Hexadiene, 3,3,5-trimethyl	C_9H_{16}	124.22
15.	7.270	0.55	2,4,6-octatriene,2,6-Dimethyl	$C_{10}H_{16}$	136.23
16.	8.199	0.10	Butanoic acid, 3-Hexenyl ester,	$C_{10}H_{18}O_2$	170.24
17.	8.548	0.08	Trans-2-Caren-4-ol	$C_{10}H_{16}O$	152.23
18.	8.911	0.06	4-Methyl-1,3-pentadiene	C_6H_{10}	82.14
19.	9.666	0.04	2-Cyclohexen-1-ol, 1-methyl-4-(1-methylethenyl)-, trans	$C_{10}H_{16}O$	152.23
20.	10.247	0.12	4-Hexyn-3-ol	$C_6H_{10}O$	98.14
21.	10.392	0.15	Bicyclo[3.1.0]hex-2-ene, 4-methylene-1-(1-methylethyl)	$C_{10}H_{14}$	134.21
22.	10.668	0.38	Alpha-Cubebene	$C_{15}H_{24}$	204.35
23.	11.046	0.25	Copaene	$C_{15}H_{24}$	204.35
24.	11.162	0.04	Bicyclo[4.1.0]heptane, 7-(1-methylethylidene)	$C_{10}H_{16}$	136.23
25.	11.263	0.87	(E, Z)-.alpha.-Farnesene	$C_{15}H_{24}$	204.35
26.	11.539	0.36	1H-Cycloprop(e)azulene, 1a,2,3,4,4a,5,6,7b-octahydro-1,1,4,7-tetramethyl-,[1aR-(1a.alpha.,4.alpha.,4a.beta., 7b.alpha.)]-	$C_{15}H_{24}$	204.35
27.	11.757	18.93	Bicyclo [6.1.0] non-1-ene	C_9H_{14}	122.21
28.	11.932	0.30	Aromandendrene	$C_{15}H_{24}$	204.35
29.	12.149	6.43	3,4-Dimethyl-2-prop-2-enyl-2,5-dihydrothiophene 1,1-dioxide	$C_9H_{14}O_2S$	186.27

Table 1: Contd.,

30.	12.367	0.97	Cycloisolongifolene	$C_{15}H_{24}$	204.35
31.	12.541	4.20	3H-Azepine, 2-methoxy-	C_7H_9NO	123.15
32.	12.658	4.69	1,4-dimethyl-8-isopropylidenetricyclo [5.3.0.0 (4, 10)]decane	$C_{15}H_{24}$	204.35
33.	12.948	1.50	4-isopropyl-1,6-dimethyl-1, 2, 3, 4-tetrahydronaphthalene	$C_{15}H_{22}$	202.33
34.	13.122	2.23	Beta Panasinene	$C_{15}H_{24}$	204.35
35.	13.340	0.39	2-Petadecen-4-yne, (Z)-	$C_{15}H_{26}$	206.36
36.	13.413	2.23	Patchoulene	$C_{15}H_{24}$	204.35
37.	13.558	0.71	Cyclooctanemethanol	$C_9H_{18}O$	142.24
38.	13.761	7.47	Caryophyllene oxide	C_7H_9NO	123.15
39.	13.950	0.66	Ledol	$C_{15}H_{26}O$	222.36
40.	14.037	2.25	o-Menth-8-ene	$C_{10}H_{18}$	138.24
41.	14.342	4.17	Adamantane	$C_{10}H_{16}$	136.23
42.	14.705	1.47	Z-3-Hexadecen-7-yne	$C_{16}H_{28}$	220.393
43.	15.010	0.75	Cedrene-V6	$C_{15}H_{24}$	204.35
44.	15.112	0.25	Cyclohexane, 1,5-diethenyl-3-methyl-2-methylene-, (1.alpha., 3.alpha.,5.alpha)	$C_{12}H_{18}$	162.27
45.	15.170	0.80	Farnesol isomer	$C_{15}H_{26}O$	222.36
46.	15.417	0.46	Bicyclo[3.2.0]heptane, 6-methylene	$C_{18}H_{24}$	110.19
47.	15.751	0.54	1-(3,3-dimethyl-1-yl)-2,2-dimethylcyclopropene-3-carboxylic acid	$C_{12}H_{16}O$	192.25
48.	16.114	0.15	Caryophyllene;	$C_{15}H_{24}$	204.35
49.	16.201	0.19	Undecan-3-one	$C_{11}H_20$	152.27
50.	16.739	0.21	1,5,5-trimethyl-6-(3-methyl- buta-1,3-dienyl)-cyclohexane	$C_{14}H_{22}$	190.32
51.	16.739	0.07	Benzoic acid, 2-propenyl ester	$C_{10}H_{10}O_2$	162.18
52.	18.293	0.04	1,6,10-Dodecatrien-3-ol, 3,7,11-trimethyl	$C_{15}H_{26}O$	222.36

Table 2: Activity of Phyto-Components Identified in the Leaves of *G. pentaphylla* by GC-MS Analysis

Sl. No	Name of the Compound	Activities
1.	Beta pinene	Allergenic, Antiinflammatory,Antiseptic, Antispasmodic, Candidicide, Flavour, Herbicide, Insecticide, Transdermal, larvicidal
2.	Camphene	Allelopathic, Antilithic, Antioxidant, Expectectorant, Flavour, Hypercholesteromic, Insectifuge, Pesticide, Spasmogenic.
3.	Gamma Terpinene	ACE-inhibitor, Acaricide, Aldose-Reductase-Inhibitors, Antiacetylcholinesterase, Antifeedant, Antioxidant, Flavour, Insectifuge, Irritant, Perfumery, Pesticide.
4.	(+) 3-Carene	Allergenic, fungicide, Irritant, Pesticide, antibacterial (Dorman and Deans, 2000).
5.	Copaene	Carminative, Antifungal
6.	Patchoulene	Antimalarial, Antiplasmoidal
7.	Caryophyllene-oxide	Antiedemic, Antifeedant, Anti-inflammatory, Antitumor, Calcium antagonist, Fungicide, Insecticide, Pesticide.
8.	Caryophyllene	Aldose-Reductase-Inhibitors, Allergenic, Analgesic, Antiacne, Antiasthamatic, antimicrobial, Anticarcinogenic, Antifeedant, Antitumor, Antiulcer, larvicide, pesticide.
9.	Nerolidol	Flavouring agent (Moser *et al*., 2001).
10.	Santolina triene	Cytotoxic, antifungal, Antibacterial, Anti-inflammatory (Muthuchelian and Ramalakshmi, 2011).

Table 2: Contd.,

11.	Undecan-3-one	Bacteriostatic (Gibka *et al.*, 2009)
12.	Terpinolene	Larvicidal (Conti *et al*., 2012); Allelochemic, Antifeedent, Antinitrosaminic, Antioxidant, Deodorant, Flavour, Perfumery, Pesticide, Cancer Chemoprevention (Okumura *et al*., 2012).
13.	Alpha terpinene	ACE-inhibitors, Acaricide, Aldose-Reductase Inhibitor, antiacetylcholinesterase, Antispasmodic, Flavour, Insecticide, P450-2B1 inhibitor, Perfumary, Spasmogenic, Repellent activity (Choi *et al*., 2002).